Recent developments in microscopical techniques for application in forest research

Terry A. Holmes and Lesley E. Manning

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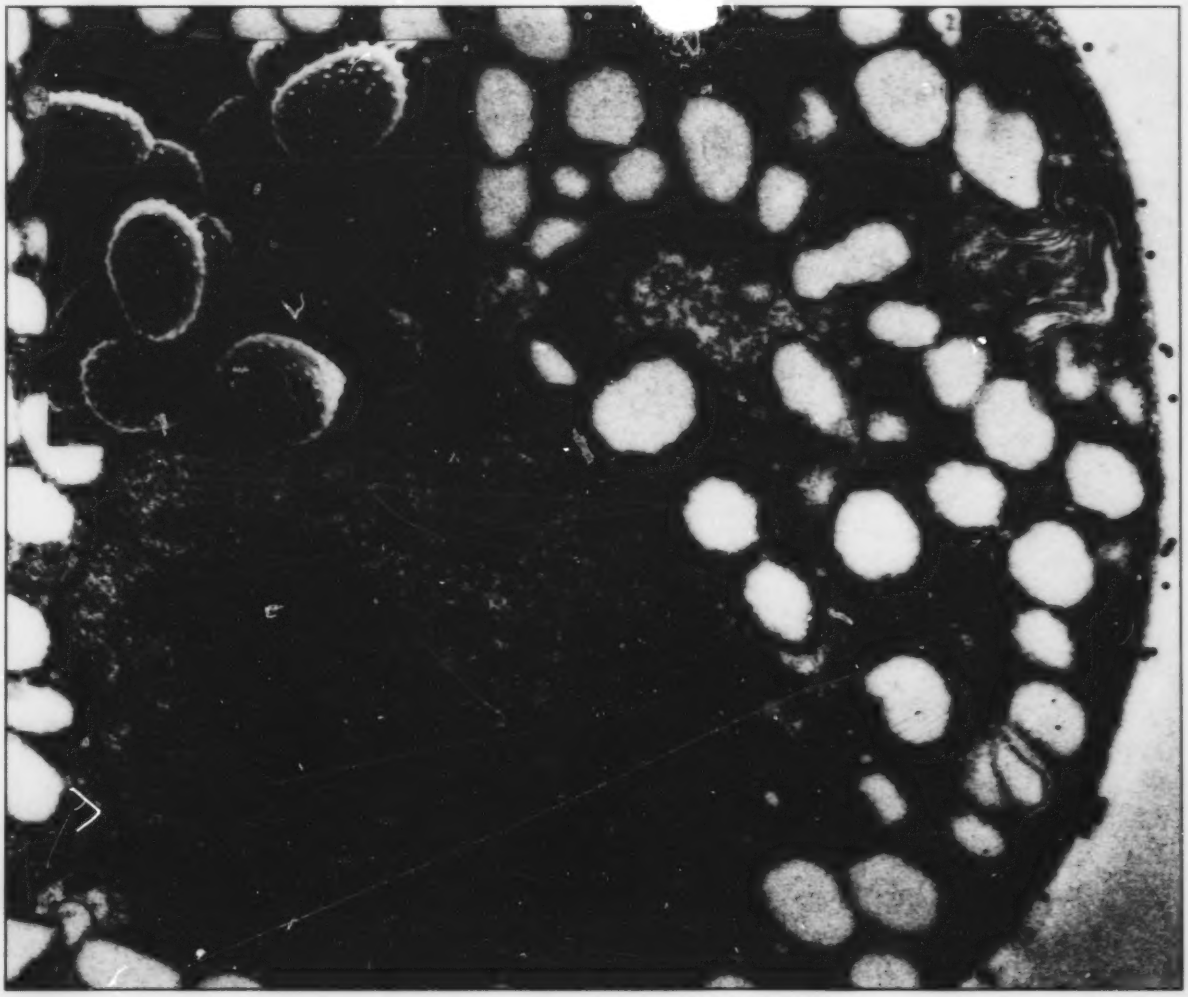

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Cover: Transmission electron micrograph of ovomucoid-gold labeled, WGA-targeted chitin in the wall of a section through a basidiospore of white pine blister rust, *Cronartium ribicola*. Inset: Scanning electron micrograph of urediospores of *Cronartium ribicola*.

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Abstract

In this report, microscopical techniques, including transmission and scanning electron microscopy, light and fluorescence microscopy, and immunocytochemistry are reviewed with examples from recent literature to illustrate prospective forestry applications. The purpose of this report is to explore potential applications of microtechniques to forest research programs in areas such as pathogen and insect management, biorational forest weed control, ecosystem dynamics, and seed and nursery research. Also included is a table of equipment currently available in the microtechnique laboratory at the Pacific Forestry Centre. Recent technical innovations and the future prospects of these techniques are discussed.

Résumé

Le présent rapport examine les techniques de microscopie, y compris la microscopie électronique à transmission et à balayage, la microscopie optique et par fluorescence et l'immunocytochimie et présente des exemples extraits de publications récentes afin d'illustrer les perspectives d'application de ces méthodes en foresterie. Ce rapport a pour but d'explorer les applications potentielles des microtechniques aux programmes de recherche forestière dans des domaines comme la répression des insectes et des agents pathogènes, la lutte biorationnelle contre la végétation concurrente en forêt, la dynamique des écosystèmes et la recherche sur les semences et en pépinière. Il présente un tableau des appareils actuellement utilisés au laboratoire de microtechnique du Centre de foresterie du Pacifique et examine les innovations technologiques récentes et leurs perspectives futures d'emploi.

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Introduction

Since the early development of electron microscopy in the 1940s, increased knowledge in tissue preparative techniques and developments in microscope technology have generated vast amounts of structural and ultrastructural information about living organisms (e. g., Klomparens 1990). Microscopy methods are now firmly established and, where necessary, modifications for specific forestry applications have been developed. This has contributed to many worthwhile developments in forest science. New forestry practices have been adopted in pest control, with the use of *Bacillus thuringiensis* [B.t.], silvicultural practices resulting from knowledge of growth and development of trees, use of pheromones from new understanding of insect morphology, and new products from research into the structure of wood. Microscopy has played an important role in each of these developments. With the pressure on forests due to declining timberlands and an increased demand for wood products, basic research is becoming more important as a valuable avenue to understanding processes in the forest ecosystem and developing mechanisms to control them for management purposes. In this endeavor, microscopy will continue to play an important role. Microscopy research studies on the efficacy of viruses, longevity of mycopesticides, effects of CO_2 levels, soil formation, and host-pathogen interactions will contribute much needed information. The objective of this review is to summarize some recent microscopical techniques with value in the forest research programs in several areas including pathogen management, insect management, biorational control of forest weeds, ecosystem dynamics, and seed and nursery services at the Pacific Forestry Centre inVictoria, B.C. All illustrations used in this review are drawn from research conducted at the Pacific Forestry Centre. Figure captions provide information on the specific research study in which the images were used.

The equipment presently available in the electron microscopy laboratory at the Pacific Forestry Centre is listed in Table 1. Microtechnique services also include paraffin-embedding equipment, as well as rotary and sliding microtomes, cryostats and pyramitomes for sectioning embedded materials. Photomicroscopes employing fluorescence, polarized light, interference contrast, and phase contrast, as well as bright field light microscopy, in both incident and transmitted modes, are available at the Pacific Forestry Centre.

The following is a selection of forestry-related papers categorized by their primary microscopical technique. As indicated in many of these papers, a combination of several microscope methods is often necessary to understand a problem. Each section includes a brief explanation of the procedures used.

Table 1. Type and function of equipment available in the electron microscopy laboratory of the Pacific Forestry Centre, Victoria, B.C.

Type	Function or Specifications
Scanning electron microscope (SEM)-JEOL	Magnification 10X to 180 000X. Equipped with 120 roll film and polaroid cameras.
Backscatter electron detector	Major attachment of the SEM that detects backscattered electrons and displays them as a composition or topographic image.
Cathodoluminescence detector	Major attachment of the SEM that detects photons emitted from the surface of a cathodoluminescent specimen. Covers visible and infrared light regions.
Transmission electron microscope (TEM)-Philips	Magnification 100 to 500 000X. Equipped with 35 mm and plate cameras.
Vacuum evaporator	Deposition of support films, carbon coating and shadow casting.
Sputter coater	Gold, gold/palladium, silver, and carbon deposition.
Critical point dryer	Preserves tissue structure by eliminating tension stresses during final solvent removal from specimens for SEM.
Cryostat	Provides a low-temperature environment (to -30°C) for sectioning frozen material.
Pyramitome	Cutting thin sections (to 1 µm) of plastic embedded tissue using glass knives.
Ultramicrotome	Cutting ultrathin sections (to 60 nm) of plastic embedded tissue using glass and diamond knives for examination with TEM.

Figure 1. Golgi apparatus in white pine needle, *Pinus monticola* (X 110 000). This image was obtained in a study of the genetics of white pine conducted by Dr. Eleanor White of the Pacific Forestry Centre.

Figure 2 Clamp connection in a Basidiomycete fungus, *Chondrostereum purpureum* in cottonwood, *Populus trichocarpa* (X 21 000). This image was obtained in a study of fungal biocontrol agents conducted by Dr. Simon Shamoun of the Pacific Forestry Centre.

Electron microscopy

The electron microscope works by producing a beam of electrons from a heated filament and accelerating the beam with high voltage toward the specimen. In the transmission electron microscope (TEM), the beam of electrons passes through an ultrathin section of the specimen and an image is viewed on a fluorescent screen. The TEM is primarily used to examine fine structure within cells and is valuable in studies of cellular functioning (Figures 1, 2 and 3).

In the scanning electron microscope (SEM), the surface of a specimen is systematically scanned with an electron beam in a raster pattern. The high energy electrons cause, among other things, the emission of secondary low energy electrons. The secondary electrons are collected and the resulting signal displayed in a synchronous raster pattern on a cathode ray tube, creating an image of the details of the specimen's surface structure. The SEM has been valuable in taxonomic studies (e. g., Figure 4), investigations of sensory organs (Figure 5) and in studies of the relation of organ structure to function, for example, the response of insects to pheromones (Figure 6). The following selected papers point out the potential value of electron microscopy in the study of various biological systems:

Johnson, J.A.; Whitney, N.J. 1989. Canadian Journal of Botany 67:3513-3516.

A study of endophytes of needles of balsam fir (Abies balsamea***) and red spruce (***Picea rubens***) in New Brunswick, Canada, using culture and electron microscope techniques***

Endophytes have the distinguishing characteristic of colonizing the interior of needles without causing obvious disease symptoms. A scanning electron microscope (SEM) was used to study the habitation characteristics of

endophytic fungi from the needles of balsam fir and red spruce. Scanning electron micrographs indicated that hyphae occupied intercellular spaces and adhered to outer wall surfaces of parenchyma cells without cell penetration.

Nanci, A.; Zalzal, S. ; Smith, C.E. 1990. The Journal of Histochemistry and Cytochemistry 38(3):403-414.

Routine use of backscattered electron imaging to visualize cytochemical and autoradiographic reactions in semi-thin plastic sections

Backscatter electron imaging (BEI) was used to examine cytochemical labeling and autoradiography in 2-µm-thick sections. BEI allowed examination of large sections and provided images with more contrast, resolution and structural details than the light microscope. Simple preparation of specimens allows examination of cell organization, structure, and content with resolution approaching that of transmission electron microscopy.

Remley, P.A.; Bradford, J.M. 1989. Soil Science Society of America Journal 53:1215-1221.

Relationship of soil crust morphology to inter-rill erosion parameters

Scanning electron micrographs (using the backscatter electron detector) and photomicrographs of thin-section soil samples were used to study surface sealing and crusting and its role in the erosion process by relating infiltration, run-off and erosion measurements to crust morphology.

Tucker, K.A.; Karnok, K.J.; Radcliffe, D.E.; Landry, G.; Roncadori, R.W.; Tan, K.H. Tan. 1990. Journal of Agronomy 82(3):549-555.

Localized dry spots as caused by hydrophobic sands on bentgrass greens

Under certain conditions, uncultivated sandy soils exhibit hydrophobicity which prevents infiltration of rain. From SEM observations, sand grains from localized dry areas were found to have an organic coating which was absent on grains from healthy areas of the green. It was concluded that the organic coating was a fulvic acid compound which becomes hydrophobic upon drying.

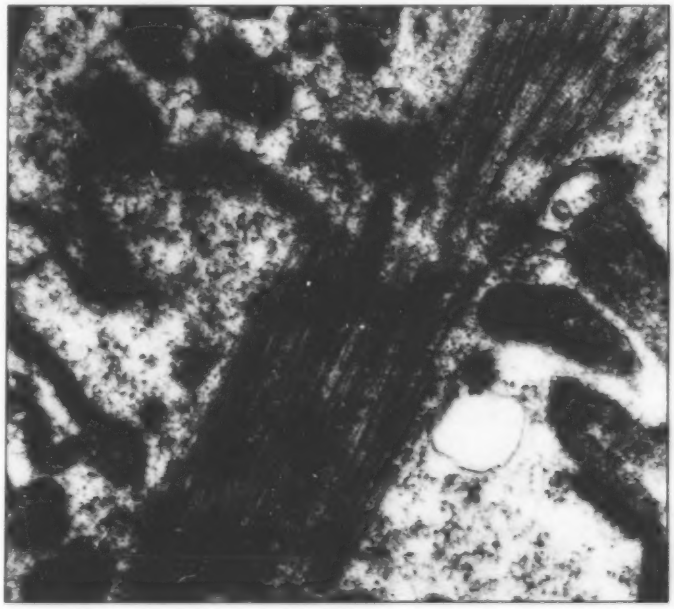

Figure 3. Wing muscle tissue in bark beetle, *Dendroctonus ponderosae* (X 50 000). This image was obtained in a study of bark beetle ultrastructure conducted by Dr. Tara Sahota of the Pacific Forestry Centre.

Osburn, J.M.; Taylor, T.N. 1990. Botanical Gazette 151(4):465-476.

Morphological and ultrastructral studies of plant cuticular membranes. I. Sun and shade leaves of Quercus velutina *(Fagaceae)*

Morphological differences are observed in broad-leaved trees developing under varying environmental conditions (e.g. sun and shade leaves). Generally, sun leaves have thicker cuticles, higher stomatal densities and have a greater

Figure 4. Predaceous dipteran, *Lonchaea corticis* (X 75). This image was obtained in a study of biological control using natural predators conducted by Dr. Michael Hulme of the Pacific Forestry Centre.

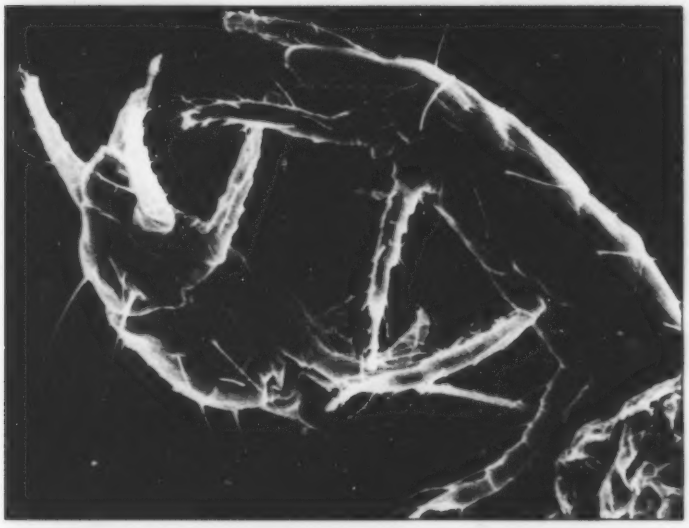

Figure 5. Male and female mite, *Pyemotes barbara* (X 325). This image was obtained in a study of mite life cycles conducted by Dr. Imre Otvos of the Pacific Forestry Centre.

Figure 6. Black army cut worm antenna, *Actebia fennica* (X 1350). This image was obtained in a study of insect sensory receptors conducted by Dr. Roy Shepherd of the Pacific Forestry Centre.

CO_2 fixing capacity. Using light microscopy, scanning and transmission electron microscopy, cuticular differences between sun and shade leaves of *Quercus velutina* at the ultrastructural and micromorphological levels were studied.

Poinar, R.H. and G.O. Poinar Jr. 1991. Journal of Invertebrate Pathology 57:137-140.

Electron microscope evidence of a new virus in the grass grub, Costelytra zealandica *(Coleoptera:Scarabaeidae)*

TEM studies were used to locate and identify a new virus within the midgut of the grass grub *Costelytra zealandica.* The potential use of this virus as a biocontrol agent is being investigated.

Staining for light microscopy

By the mid 1800s, light microscope optics had been improved to near the theoretical limit. However, the lack of image contrast remained a major impediment to the use of microscopes for biological investigation. This was solved in 1854 when the use of staining materials to increase contrast was demonstrated by Hartig (Bracegirdle 1989). Staining techniques are now available to distinguish specific cellular structures, tissue types, and developmental stages, and to differentiate between various organisms. Staining has become highly specific for certain tissues and cellular functions by immunocytological techniques (see the section on immunocytochemistry).

Krishna Moorthy, P.N.; Alexander, M.P.;Tewarp, G.C. 1988. Journal of Economic Entomology 81(1):403-405.

Versatile method for staining insect eggs and larvae in leaves

To determine the appropriate time for insect control measures, knowledge of pest oviposition behavior is essential. In this report, a rapid technique for staining insect larvae and eggs within leaf tissue is described.

Jennings, D.M., Ford-Lloyd, B.V. and G.M. Butler. 1989. Mycological Research 92(2):230-232.

An aniline blue squash technique for the observation of urediniospore germ pores

Many rust species have indistinct germ pores and the number and position of urediniospore germ pores are used as taxonomic characteristics. A staining technique using aniline blue is described that is useful for identifying rust germ pores.

Koske, R.E.; Gemma, J.N. 1989. Mycological Research 92(4):486-488.

A modified procedure for staining roots to detect VA mycorrhizas

The fungi forming vesicular-arbuscular (VA) mycorrhizas are obligate symbionts. VA mycorrhizas are the only endomycorrhizas found in conifers. This modified staining technique for the detection of VA mycorrhizal fungi eliminates many toxic compounds commonly used in root fixation/staining procedures without reducing the resolution of staining.

Fluorescence microscopy

One of the most important developments in the history of microscopical observation was the treatment of tissues for fluorescence. This is summarized by Haitinger (1938) cited in Bracegirdle (1989). Fluorescence is the emission of light at a certain wavelength when a structure is irradiated by light of a shorter, more energetic wavelength. This can result from an inherent property or from a fluorochrome being bound to a specific

cellular site. The technique is useful for delineating developmental stages and tissue types, and to distinguish between different organisms.

Swanson, S.B.; Yarrow, S.A.; Coumans, M.P.; Erickson, L.R. 1990. Stain Technology 65(5):251-258.

Vital fluorescent staining technique for microspores of Brassica napus

By contrasting the exine, cell wall/intine, and nucleus with three fluorescent stains, the ontogeny of microspore-derived embryo development in *Brassica napus* was followed.

Pfender, W.F.; King, G.L.; Rabe , J.R. 1991. Phytopathology 81:109-112.

Use of dual-stain fluorescence microscopy to observe antagonism of Pyrenophora tritici-repintis *by* Limonomyces roseipellis *in wheat straw.*

To understand the mechanisms of antagonism between two fungi, a technique using an antibody-based fluorescent stain and a lectin-conjugated stain (see the section on immunocytochemistry) was developed that allows visualization of the hyphae within host tissue while distinguishing between the two species of fungus.

Stelly, D.M.; Kautz, K.C.; Rooney, W.L. 1990. Crop Science 30:952-955.

Pollen fertility of some simple and compound translocations of cotton

Pollen semisterility is useful for detecting cytogenetic deficiencies and aberrations in diploid crops. A fluorochrome reaction method for the detection of cytogenetically induced semisterility in cotton is described.

Ojeda, J.L.; Angeles Ros, M.; Icardo, J.M. 1989. Stain Technology 64(5):243-248.

A technique for fluorescence microscopy in semithin sections

Fluorescence microscopy is especially advantageous when used with lectins and antibodies. Unfortunately, images are often degraded due to scattered or emitted light from outside the focal plane. A method to overcome these difficulties and obtain high-contrast and high-resolution fluorescent images using semithin sections is described.

Firstencel, H.; Butt, T.M.; Carruthers, R.I. 1990. Journal of Invertebrate Pathology 55:258-264.

A fluorescence microscopy method for determining the viability of entomophthoralean fungal spores

Several environmental factors affect fungal spore survival. In this study, the potential use of fluorochrome stains to assess the viability of entomophthoralean species was presented. When compared to standard germination tests, fluorescein diacetate and propidium iodide proved accurate and precise for determining viability of certain fungal species.

Brammall, R.A. ; Higgins, V.J. 1988. Canadian Journal of Botany 66:915-925.

A histological comparison of fungal colonization in tomato seedlings susceptible or resistant to Fusarium *crown and root rot disease*

Inoculated seedling cultivars resistant and susceptible to *Fusarium* root rot were histologically compared for the rate and extent of primary root colonization. To determine infection progress, samples were taken daily, fixed, and examined by epifluorescence and Nomarski interference contrast microscopy. Seventy-two hours after inoculation, phenolic and lignin-like compounds were associated with the cortical wall, which appears to be important as a structural defense barrier.

Figure 7. Colloidal gold labeled urediospore of *Cronartium ribicola* (X 50 000). This image was obtained in a study of fungal spore ultrastructure conducted by Dr. Abul Ekramoddoullah of the Pacific Forestry Centre.

Figure 8. Colloidal gold labeled bark beetle stomach, *Dendroctonus ponderosae* (X 27 000). This image was obtained in a study of insect ultrastructure conducted by Dr. Tera Sahota of the Pacific Forestry Centre.

Immunochemistry

In recent years, a number of cytochemical techniques have been developed to identify, localize and quantify cell components (Horisberger 1981). Cytochemical techniques allow microscopists to locate and quantify sites which have a particular physiological function within the cell (Figures 7 and 8). Immunocytochemical techniques provide increased specificity by employing an antibody which is labeled with gold or a fluorochrome to recognize a unique active site in the tissue. This binding site is readily discernible with an electron or light microscope respectively. This informaton enhances an understanding of basic life processes such as resistance to disease and inheritance patterns.

Danscher, G.; Rytter Nörgaand, J.O. 1983. The Journal of Histochemistry and Cytochemistry 31(12):1394-1398.

Light microscope visualization of colloidal gold on resin-embedded tissues

A technique is described by which 1-µm-thick sections of tissue embedded in resin are labeled with an enzyme-gold complex and further enhanced for light microscopy by encapsulating the gold particles in silver using photographic developer. This technique may also be carried out on frozen and paraffin-embedded sections and counterstained (Danscher 1981a and 1981b).

Hardham, A.R.; Suzaki, E. 1990. Canadian Journal of Microbiology 36:183-192.

Glycoconjugates on the surface of the pathogenic fungus Phytophthora cinnamomi *studied using fluorescence and electron microscopy and flow cytometry*

Using lectins labeled with fluorescent and electron-dense probes, surface glycoconjugates of zoospores and cysts of the fungus *Phytophthora cinnamomi*, and their role in the infection of plants, was investigated.

Benhamou, N.; Grenier J. ; Asselin, A. 1991. Physiological and Molecular Plant Pathology 38:237-253.

Immunogold localization of pathogenesis-related protein P14 in tomato root cells infected by Fusarium oxysporum f. radicis-lycopersici

Immunogold cytochemical techniques were utilized for subcellular location of pathogenesis-related proteins in tomato roots. Accumulation of protein P14 in host cell walls of resistant varieties indicates a mechanism for resistance to this pathogen.

Mustardy, L.; Cunningham, F.X. ; Gantt, E. 1990. Plant Physiology 94:334-340.

Localization and quantitation of chloroplast enzymes and light-harvesting components using immunocytochemical methods

Seven chloroplast proteins were localized in the red alga *Porphyridium cruentum* using immunoelectron microscopy. This technique provided a quantitative estimation of photosynthetic components which could not be quantified by other methods such as immunoelectrophoresis or immunoblotting.

Suske, J. and G. Acker. 1988. Canadian Journal of Botany 67:1768-1774.

Identification of endophytic hyphae of Lophodermium piceae *in tissues of green, symptomless Norway spruce needles by immunoelectron microscopy*

In electron microscope observations of the ultrastructural interaction of an endophyte and its host, identification problems often arise due to multiple infections. These authors used antiserum specific for *L. piceae* and an on-section immunogold labeling technique which provides clear structural information of the relationship between host and endophyte and leads to an understanding of the roles of these fungi in the needle tissue.

Blanchette, R.A.; Abad, A.R.; Farrell, R.L. ; Leathers, T.D. Leathers. 1989. Applied and Environmental Microbiology 55(6):1457-1465.

Detection of lignin peroxidase and xylanase by immunocytochemical labeling in wood decayed by basidiomycetes

The ultrastructural localization of some of the major enzymes involved in lignin degradation (lignin peroxidase H8 and H2, and xylanase), by using colloidal-gold immunocytochemistry, in birch wood decayed by three white rot fungi (*Phellinus pini, P. chrysosporium* and *Trametes versicolor*) and one brown rot fungus (*Fomitopsis pinicola*) is reported. Studies of this nature are valuable in producing an accurate picture of where and when the major enzymes involved during the decay process of wood occur.

Blanchette, R.A.; Abad, A.R.; Cease, K.R.; Lovrien, R.E.; Leathers, T.D. 1989. Applied and Environmental Microbiology 55(9):2293-2301.

Colloidal gold cytochemistry of endo-1,4-fl-glucanase, 1,4-fl-D-glucan cellobiohydrolase, and endo-1,4-fl-xylanase: ultrastructure of sound and decayed birch wood

Colloidal gold immunocytochemistry was used to determine the ultrastructural localization of cellulose and xylan in uninfected birch wood and the distribution of cellulose and xylan after decay by three white rot fungi (*Phellinus pini, P. chrysosporium* and *Trametes versicolor*) and one brown rot fungus (*Fomitopsis pinicola*).

Spiegel, Y.; McClure, M.A.; Kahane, I.; Robertson, W.M.; Salomon, R. 1991. Journal of Nematology 23(4):451-456.

Wheat germ agglutinin bound to the outer cuticle of the seed gall nematodes Anguina agrostis *and* A. tritici

Host specificity exhibited by nematodes and their dependence on host plant development suggests that recognition may be important for host-parasite compatibility. Maturation of *Anguina* coincides with lectin synthesis in wheat and ryegrass seed formation. Wheat germ agglutinin (WGA) binding sites occur on the cuticular surface of juvenile nematodes. In this study, the occurrence and origin of WGA was investigated using fluorescent labeled antibodies.

Recent innovations in microscopy

Recent progress in microscopy demonstrates that improvement of traditional optical and electron microscope designs is far from exhausted (Howie 1989). The optical microscope and the transmission and scanning electron microscopes have each become the basis for an expanding technology, e.g., low-temperature scanning electron microscopy, scanning electron energy dispersive X-ray analyzer, and most recently, scanning tunneling microscopy (STM)(Hansma 1988).

The following citations are an introduction to new technologies which have definite applications to forestry research.

Login, G.R.; Dwyer, B.K.; Dvorak, A.M. 1990. The Journal of Histochemistry and Cytochemistry 38(6):755-762.

Rapid primary microwave-osmium fixation. Preservation of structure for electron microscopy in seconds

Using a microwave source (oven), fine structural preservation of tissue blocks was achieved in aldehyde and/or osmium within seconds as opposed to hours. Reducing the time that a sample is kept in primary and post-fixative solutions can permit preservation of cell constituents which are otherwise lost in long fixation procedures; it can also preserve protein antigenicity, especially in the case of low molecular weight, soluble proteins such as enzymes which tend to diffuse from one cell compartment to another during traditional fixation procedures.

Charlton, W.A.; Macdonald, A.D.; Posluszny, U.; Wilkins, C.P. 1989. Canadian Journal of Botany 67:1739-1743.

Additions to the technique of epi-illumination light microscopy for the study of floral and vegetative apices

Various stains designed for epi-illumination light microscopy were used in conjunction with a cryo-SEM to observe morphogenesis of meristems with a greater depth of field and resolving power than the light microscope.

Feijtel, T.C.; Jongmans, A.G.; van Doesburg, J.D.J. 1989. Soil Science Society of America Journal 53:876-882.

Identification of clay coatings in an older quaternary terrace of the Allier, Limagne, France

Using a scanning electron microscope energy dispersive x-ray spectrometer, elemental composition of clay coatings was obtained.

Smith, W.H.; Pooley, A.S. 1989. Forest Science 35(4): 1114-1124.

Red spruce rhizosphere dynamics: spatial distribution of aluminum and zinc in the near-root soil zone

Forest soils represent important sinks for heavy metals associated with air pollution. The uptake by roots of naturally occurring metals, such as aluminum, may be altered due to changes in soil chemistry. In this study, roots with associated rhizosphere soil were freeze dried and examined with a scanning electron microscope and an energy dispersive x-ray spectrometer to investigate the importance of the forest rhizosphere in root uptake of heavy and trace metals.

Figure 9. Colloidal gold labeled fungal hyphae in pine needle, *C. ribicola* in *Pinus lambertiana* (X 27 500). This image was obtained in a study of host pathogen interaction conducted by Dr. Abul Ekramoddoullah and Garry Jensen of the Pacific Forestry Centre.

Figure 10. Mycorrhiza, spruce, *Picea glauca* root with *Laccaria* sp. (X 5000). This image was obtained in a study of mycorrhizal root systems conducted by Dr. Tony Trofymow of the Pacific Forestry Centre.

Figure 11. Decaying wood (X 28). This image was obtained in a study of forest soil formation conducted by Dr. Caroline Preston of the Pacific Forestry Centre.

Figure 12. Stomatal distribution on western red cedar, *Thuja plicata*, under high CO_2 levels (X 105). This image was obtained in a study of the effect of carbon dioxide on stomatal distribution conducted by Dr. Al Mitchell of the Pacific Forestry Centre.

Future prospects

Techniques are continually being developed in microscopy. These will produce valuable information in many areas of forestry research. The use of this information is expected to improve forestry practices and forest products as it has in the past. Forestry research areas which can benefit from microscope investigations include biopesticides, host-pathogen interactions to either enhance resistance mechanisms in crop plants or augment natural pathogens on weed species (Figure 9) , fertilization and mycorrhizal (Figure 10) impact on tree growth, soil processes (Figure 11), impact of logging practices, soil population dynamics, impacts of silvicultural treatments, and climate change (e.g. impacts of elevated CO_2 levels on growth and development of commercial species) (Figure 12). At the Pacific Forestry Centre, computer enhancement of digitized images from our electron microscopes will soon be possible with the retrofitting of a "gated integrator" being developed by several companies. *In situ* hybridization for the localization of messenger RNA using cDNA as probes is being developed in several laboratories and will undoubtedly acquire increased applicability and relevance as a complement to immunocytochemistry in the next few years. For example, investigations of genetic control of plant tissue development will benefit from this technique.

Acknowledgements

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